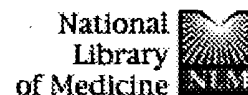


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



















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
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
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
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
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
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
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
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
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
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
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
















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

















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
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
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
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
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







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
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
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
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
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
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
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
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
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
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
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


















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
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
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
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
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
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
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















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
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
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
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
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
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
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
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
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
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


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
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
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
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
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
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
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
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
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
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
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















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



















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
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
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




































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
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
















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















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
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
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
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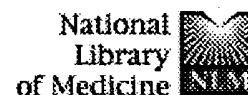
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

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

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









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TI Notch ligands and their role in the proliferation and fate of
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AU Mori, A. [Reprint Author]; Schwartz, P. H.; Palmer, T. D. [Reprint Author]

CS Dept. Neurosurgery, Stanford Univ, Stanford, CA, USA

SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003)

Vol. 2003, pp. Abstract No. 562.9. <http://sfn.scholarone.com>. e-file.

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TI ***HUMAN*** ***NEURAL*** ***PROGENITOR*** CELLS SHOW NORMAL
VENTROLATERAL MIGRATION IN ORGAN CULTURES OF HUMAN EMBRYONIC
MESENCEPHALON.

AU Jarman, R. G. [Reprint Author]; Freed, C. R. [Reprint Author]

CS Div Clin Pharm, Dept of Med, Univ Colo Sch of Med, Denver, CO, USA

SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002)

Vol. 2002, pp. Abstract No. 726.7. <http://sfn.scholarone.com>. cd-rom.

Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience.

Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.

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TI Induction of tyrosine hydroxylase expression in growth factor expanded
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author]; Juliusson, B. [Reprint author]; Seiger, A.; Wahlberg, L. U.
[Reprint author]

CS Cellular Biology, NsGene A/S, Ballerup, Denmark

SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 2, pp. 1821.
print.

Meeting Info.: 31st Annual Meeting of the Society for Neuroscience. San Diego, California, USA. November 10-15, 2001.

ISSN: 0190-5295.

DT Conference; (Meeting)

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TI Site-specific neuronal differentiation of growth factor expanded
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transplantation to the neonatal rat brain.AU England, U. [Reprint author]; Fricker, R. A. [Reprint author]; Carpenter,
M. K.; Jackson, J.; Sherman, S.; Wictorin, K. [Reprint author]; Bjorklund,
A. [Reprint author]

CS Wallenberg Neurocentrum, Lund Univ., Lund, Sweden
SO Society for Neuroscience Abstracts, (1999) Vol. 25, No. 1-2, pp. 213.
print.
Meeting Info.: 29th Annual Meeting of the Society for Neuroscience, Part
1. Miami Beach, Florida, USA. October 23-28, 1999. The Society for
Neuroscience.
ISSN: 0190-5295.

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AU Carpenter, Melissa K. [Reprint author]; Cui, Xia; Hu, Zhong-yi; Jackson,
Jennifer; Sherman, Sandy; Seiger, Ake; Wahlberg, Lars U.
CS Geron Corporation, 230 Constitution Dr., Menlo Park, CA, 94025, USA
SO Experimental Neurology, (Aug., 1999) Vol. 158, No. 2, pp. 265-278. print.
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FI US 2003203844 20031030

DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION

CLMN 106
GI 35 Figure(s).

FIG. 1 depicts the effect of PDGFs on proliferation of cultured,
non-adherent mouse neurospheres.

FIG. 2 shows the effect of PDGFs on proliferation of adherent cultured
mouse NSC/progenitor cells.

FIG. 3 is western blots showing the effect of PDGF-AA in cultured
neurospheres. Downregulation of GFAP (left); upregulation of beta-III
Tubulin (right).

FIG. 4 depicts the effect of PDGF-AA in cultured mouse NCCs/ neural
progenitor cells (NPCs). Upper panel: adult mouse NSCs treated with
PDGF-AA for 6 days switch from an undifferentiated (left panel) to a
neuronal phenotype (right panel) increasing the specific neuronal marker
beta-III Tubulin. Lower panel: in contrast to the above, adult mouse NSCs
treated with PDGF-AA for 6 days significantly decrease the specific
expression of the glial specific marker GFAP indicating that the
astroglial component was reduced in presence of PDGF-AA.

FIG. 5 shows the effect of PDGF-AA and PDGF-BB on neuronal differentiation
of adult mouse stem cells. Compared to the control (panel A) the
stimulation with PDGF-AA (panel B) and PDGF-BB (panel C) significantly
increase the number of betaIII Tubulin positive cells in culture.

FIG. 6 is a Western blot showing the effect of PDGF-AA and PDGFBB on
neuronal differentiation of adult mouse stem cells. Compared with the
control (A) the stimulation with PDGF-AA (B) and PDGF-BB (C)
significantly increased levels of beta-III Tubulin protein. Sample loads
were normalized.

FIG. 7 represents the effect of PDGF-BB on the number of BrdU positive
cells in the dentate gyrus.

FIG. 8 shows the effect of PDGF-BB on BrdU positive cells in the striatum.

FIG. 9 depicts a large neuron in the MPTP-lesioned mouse receiving PDGF.
This tyrosine hydroxylase-positive (brown) nerve cell with a violet
nucleolus in the centrally placed nucleus without brown staining had an
estimated volume of 10, 900 mu m3. Anti-tyrosine hydroxylase was

visualized with the avidin-biotin-peroxidase-DAB method and cresyl violet was employed as counterstain. Bar=10 μ m.

FIG. 10 represents a small tyrosine hydroxylase-positive neuron in the MPTP-lesioned PDGF-treated mouse. The cell had an estimated volume of 170 μ m³, e.g. similar to a small glia cell, but demonstrated a clear neuronal bipolar phenotype with long dendrites that extended for more than 100 μ m in the 40 μ m thick section.

FIG. 11 shows a 3H thymidine label (black dots) over a substantia nigra neuron (Nissl stain) in a PDGF-treated MPTPlesioned mouse. Bar=10 μ m.

FIG. 12 shows that PDGFR-A and PDGFR-B genes are expressed in cultured human neural stem cells.

FIG. 13 shows immunohistochemically stained BrdU-labeled cells in the striatum at 5 weeks after PDGF infusion. (A) PBS control, (B) PDGF treated.

FIG. 14 represents the number of BrdU-labeled cells in the striatum at 12 days and 5 weeks after PDGF or BDNF infusion. *p less-than 0.05 compared to PBS infused controls. Means \pm SEM.

FIG. 15 depicts the number of BrdU/NeuN double-labelled cells in the striatum at 5 weeks after PDGF or BDNF infusion. Note that due to heterogeneity of variance with groups, data were logarithmically transformed. *p less-than 0.05 compared to PBS infused controls. Means \pm SEM.

FIG. 16 shows the number of BrdU-labeled cells in the substantia nigra at 12 days and 5 weeks after PDGF or BDNF infusion. *p less-than 0.05 compared to PBS infused controls. Means \pm SEM.

FIG. 17 depicts neurogenesis in the hippocampus which is characterized by proliferative clusters of cells along the border between the granule cell layer (g) and the hilus region (h). These cells begin to migrate into the granule cell layer about 1 week after their last cell division and can be colabeled with markers for granule cells (e.g., NeuN and Calbindin).

FIG. 18 represents Flk-1-positive cells in the dentate gyrus. Frequently Flk-1 positive cells are associated with clusters of proliferating cells. These clusters contain endothelial cells as well as NSCs/NPCs. (A) Multiple immunofluorescence with BrdU, VEGF and Flk-1. Note the colocalization of Flk-1 and BrdU. (B) BrdU positive cells are associated with blood vessels as shown with RECA staining (rat endothelial cell antigen).

FIG. 19 shows the localization of Flk-1 immunoreactive cells in the ventricle wall. The ependymal layer of the ventricle wall shows intense immunoreactivity for Flk-1. Since neural stem cells can be generated from ependymal cells of the ventricle wall, Flk-1 could function as a stem cell marker and has perhaps a function in stem cell recruitment.

FIG. 20 represents intracerebroventricular infusion of VEGF. VEGF was infused via osmotic minipumps for 7 days into the lateral ventricle of adult rats. Increased BrdU labeling in the granule cell layer of the dentate gyrus is observed 4 weeks after infusion of VEGF into the lateral ventricle.

FIG. 21 shows VEGF mRNA levels. Neurospheres were cultured in a defined medium in the presence of EGF and FGF. Using RT-PCR, the mRNA from these cells was examined for the expression of 4 different isoforms of VEGF. GAPDH expression served as quality control of the mRNA.

FIG. 22 shows Flk-1 mRNA levels. Left. Neural stem cells from the lateral ventricle wall (LV) and hippocampus (HC) of the adult rodent can be grown as neurospheres using defined medium and the growth factors ***FGF*** - ***2*** and EGF. Right. From spheres under growth condition mRNA was isolated and RT-PCT for Flk-1 was performed. Actin was used as a control for mRNA amount.

FIG. 23 shows the release of VEGF protein. Competitive ELISA for quantification of VEGF-secretion was used on adult NSCs from the rat lateral ventricle wall. As a positive control, the endothelial cell line HMEC-1 and primary retinal pigment epithelial cells (RPE) were used.

FIG. 24 depicts VEGF-dependent proliferation of NSCs. Neurosphere cultures were grown in serum free medium containing EGF/ ***FGF*** - ***2***. VEGF was added to this medium in different concentrations for 7 days and the total number of NSCs was counted.

FIG. 25 shows that the effect of VEGF is modulated via the Flk-1 receptor. NSC cultures were stimulated for 7 days with different concentrations of VEGF-A165 (triangle) and VEGF-A121 (squares). VEGF-A165 bind to all VEGF-receptors whereas VEGF-A121 binds preferentially to the Flk-1 receptor.

FIG. 26 shows the lineage potential of clonally-derived rat neural stem cell cultures. Individual clones derived from rat lateral ventricle wall cultured on poly-ornithin/laminin matrix were differentiated in NB/B27 medium supplemented with 1% FCS for 7 days and immunostained for the presence of (a) neurons with BIII-tubulin, (b) ***astrocytes*** with GFAP or (c) oligodendrocytes with GalC (bar=40 μ m).

FIG. 27 is a Dose-Response-Curve for VEGF in rat neural stem cells from the adult lateral ventricle wall. The dose-response curve was performed on clonally derived neural stem cells. Maximal growth activity can be observed starting at 50 ng/ml.

FIG. 28 (A) In basal medium VEGF (50 ng/ml) stimulates the expansion of neural stem cell cultures about 5-fold. The VEGF receptor inhibitors PADQ and SU1498 are both able to block the VEGF response at concentrations specific for VEGF receptor flk1. Total cell counts at 7 days after treatment. (B) In growth conditions (including EGF and ***FGF*** - ***2***), VEGF stimulates the expansion of neural stem cell cultures about 2-fold. The VEGF receptor tyrosine kinase inhibitors PADQ and SU1498 are both able to block the VEGF response. Total cell counts at 7 days after treatment in growth medium

FIG. 29 shows VEGF production and release into the cell culture medium. Cells were cultured in serum free medium and medium was collected at 2 days (2 d) or 8 days (8 d) in medium. HUVEC: Human umbilical vein endothelial cells, hURPE: human retinal pigment epithelial cells.

FIG. 30 represents NSCs under growth conditions (with EGF and ***FGF*** - ***2***) VEGF-receptor-Tyrosine kinase receptor blocker PADQ and SU1498 are both able to significantly reduce the growth of neural stem cell cultures. DMSO was used to dissolve the inhibitors and had no effect on the growth rate. Total cell counts at 7 days after treatment.

FIG. 31 shows that BrdU incorporation is increased under VEGF and reduced under VEGF receptor blockade. Neural stem cell cultures were treated with 50 ng/ml VEGF, 100 nM PADQ or 700 nM SU1498 for 7 days. BrdU (10 μ M) was added to the culture medium 24 hrs before cells were harvested and lysed. DNA was extracted and BrdU content was determined using an anti-BrdU ELISA. The data are presented as percent changes in optical density compared to control (Growth medium).

FIG. 32 shows that DNA fragmentation is increased under VEGF and reduced under VEGF receptor blockade. Neural stem cell cultures were treated with 50 ng/ml VEGF, 100 nM PADQ or 700 nM SU1498 for 7 days. BrdU (10 μ M) was added to the culture medium 24 hrs before cells were harvested and lysed. DNA was extracted and BrdU content was determined using an anti-BrdU ELISA. The data are presented as percent changes in optical density compared to control (Growth medium).

FIG. 33 represents in vitro generation of spheres is stimulated by intraventricular VEGF infusion. After 7 days of intraventricular infusion of either artificial cerebrospinal fluid (CSF) or VEGF, cells were isolated from the lateral ventricle wall, seeded at 10000 cells/well in Growth medium (Neurobasal+B27+EGF+ ***FGF*** - ***2*** +heparin) and grown in culture for 3 weeks. The efficiency to generate spheres from the lateral ventricle wall is substantially increased by previous in vivo infusion of VEGF. It was concluded from this finding, that VEGF stimulates the multipotent neural stem cells of the lateral ventricle wall in vivo leading to a facilitated neural stem cell growth in vitro.

FIG. 34 shows that the VEGF and FLT-4 genes are expressed in cultured human neural stem cells.

FIG. 35 shows that the FLT-1 and FLK-1 genes are expressed in cultured human neural stem cells.

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BDNF-RESPONSIVE POPULATION OF NEURAL AND NEURONAL PROGENITOR CELLS IN THE
ADULT HUMAN FOREBRAIN; THERAPY FOR BRAIN DISORDERS
IN GOLDMAN STEVEN A; NEDERGAARD MAIKEN
PA Unassigned Or Assigned To Individual (68000)
PI US 2003049234 A1 20030313
AI US 1999-271969 19990318
PRAI US 1998-79226P 19980325 (Provisional)
FI US 2003049234 20030313
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
CLMN 41
GI 6 Figure(s).

FIG. 1 shows that cultures of the adult rat SZ typically display a burst of neuronal outgrowth during the first 11 days in vitro (DIV) but suffer virtually complete neuronal loss during the 2 weeks thereafter. However, in the presence of BDNF (20 ng/ml), new adult neurons survived substantially longer than did their counterparts grown in unsupplemented media. FIG. 1A shows adult rat SZ neurons grown in control medium after 22 days. FIGS. 1B and 1C show sister cultures grown in BDNF after 36 and 57 days in vitro, respectively.

FIG. 2 shows a region sampled for culture and immunohistology. FIGS. 2A-B

are pre- and post-operative coronal MRI images of a 37 year-old female with refractory epilepsy and radiographic mesial temporal sclerosis. FIG. 2A shows atrophic sclerosis of the right hippocampus and dentate gyrus (arrow). FIG. 2B is of the same patient, 2 weeks after surgical resection. FIG. 2C shows a schematic coronal view of temporal lobe, showing the subventricular region typically sampled for culture. Cortical specimens were typically obtained from the adjacent inferior temporal gyrus (modified with permission from Kirschenbaum et al., 1994 (Kirschenbaum, 1994, which is hereby incorporated by reference)).

FIG. 3 shows that human temporal SZ-derived neurons are physiologically active and are the product of neurogenesis in vitro. A-F, cultures were challenged with high K^+ to seek evidence of neuron-like depolarization-induced increments in cytosolic calcium (Ca^{2+}_i). In this plate, a temporal SZ culture was tested after 28 days in vitro, after loading with the Ca^{2+} -sensitive dye fluo-3. FIG. 3A is a phase micrograph of two adjacent cells, one neuron-like and the other astrocytic. Scale bar=25 μm . FIG. 3B shows their baseline levels of Ca^{2+}_i , as viewed by confocal microscopy with laser scanning at 488 nm. FIG. 3C depicts the same two cells within seconds after exposure to 60 mmol/ K^+ . The neuron-like cell increased its Ca^{2+}_i rapidly and reversibly, in contrast to the cocultured ****astrocytes****. FIG. 3D shows that with the addition of tetrodotoxin (TTX) (1 $\mu mol/L$; Sigma Chemical Co., St. Louis, Mo.), K^+ -stimulation yielded a more than sixfold rise in neuronal cytosolic Ca^{2+}_i , whereas astrocytic Ca^{2+}_i increased less than twofold. The depolarization-induced Ca^{2+} increment of this cell suggested its neuronal phenotype, as did the TTX accentuation of its Ca^{2+}_i response. The increased density of TTX-sensitive Na^+ channels in neurons, relative to glia, would have been expected to yield a neuron-selective enhancement of the K^+ -stimulated Ca^{2+}_i response by TTX. FIG. 3E, at withdrawal of K^+ from the medium, each cell returned to its resting Ca^{2+}_i level. FIG. 3F, after addition of the calcium inophore lasalocid (50 mmol/L, Sigma), added as a positive control to maximize Ca^{2+} entry in both cells. These results suggested the activity of voltage-gated calcium channels in the adult-derived neurons. FIGS. 3G and 3H show cultures which were stained for neuron-specific antigens and were subjected to 3H-thymidine autoradiography. FIG. 3G depicts a MAP-5+ cell observed in dissociate culture after 15 days in vitro. FIG. 3H shows a cell which has incorporated 3H-thymidine in vitro, suggesting its origin from precursor mitosis (Kirschenbaum, 1994, which is hereby incorporated by reference).

FIG. 4 demonstrates that the adult human subependyma harbors scattered islands of apparent progenitor cells. The ependyma/ subependyma lining the temporal horn of the lateral ventricle, deep to the inferior temporal gyrus, in a 27 year-old man with mesial temporal sclerosis is shown. This region had distinct squamous ependymal and cuboidal subependymal layers, each 1-2 cells deep. FIGS. 4A-B show scattered islands of subependymal cells expressed the neural progenitor cell marker musashi. These sections were immunoperoxidase stained for musashi using anti-mouse musashi IgG. FIGS. 4C-D show loose aggregates of adult SZ cells also expressed Hu proteins, a triad of early, neuron-specific RNA-binding proteins recognized by Mab 16A11 (Baram, et al., 1995; Szabo, et al. 1991, which are hereby incorporated by reference). Immunostaining revealed frequent pockets of Hu+ scattered about the ventricular epithelium (Scale=50 μm).

FIG. 5 demonstrates that serial treatment with fibroblast growth factor-2 ("FGF2") and brain-derived neurotrophic factor ("BDNF") allows for the expansion and survival of neurons arising from human subependymal zone ("SZ"). FIG. 5A shows cell outgrowth from adult human SZ, 9 wks in vitro. The explant was exposed to FGF2 for a week, in the presence of 3H-thymidine, then to BDNF for 2 months, then fixed and stained for the neuronal marker MAP-2. FIG. 5B shows that two of these MAP-2+ neurons incorporated 3H-thymidine during their first week in vitro (aggregations of silver grains denoted by arrows), indicating mitotic neurogenesis during the period of FGF2 exposure. FIG. 5C is a high power visualization of the cell asterisked in FIGS. 5A-B, stained for MAP-2. FIG. 5D shows the cells after 3Hthymidine autoradiography. FIG. 5E is another outgrowth from an adult temporal SZ explant, 7 weeks in culture. This sample was also raised in FGF2 followed by BDNF, then subjected to confocal imaging of the fluorescence signal emitted by the calcium indicator dye fluo-3; this was done to assess neuronal responses to depolarizing stimuli. FIG. 5F depicts the baseline fluo-3 fluorescence signal from the neurons indicated in phase in 5E (arrows). FIG. 5G was taken immediately after depolarization by 60 mM KCl. A similar response to 10 μM glutamate was observed. In each of these photos, the Scale=25 μm .

FIG. 6 shows that rare neocortical explants also exhibited neuronal production and outgrowth. In cultures derived from 2 patients, neuronal outgrowth was noted from explants derived from both the SZ and cortex.

This suggested that temporal cortex might harbor residual neuronal precursors, analogous to those of the SZ. FIG. 6A shows a field of neurons found in a temporal neocortical explant culture after 9 wks in vitro, revealing a congregation of neurons lying upon a field of ependymal cells and glia. This culture was derived from a 28 year-old man, and was treated sequentially with ***FGF*** - ***2*** (1 wk) and BDNF (8 wks). The asterisk in FIG. 6A corresponds to the same position as that in FIGS. 6B-E. FIG. 6B is a higher magnification within FIG. 6A (note: FIGS. 6B-E are rotated 90 degrees counter-clockwise with respect to FIG. 6A). FIG. 6C demonstrates that immunostaining for MAP-2 indicates the neuronal identity of these cells. FIG. 6D provides the baseline calcium signal of these neurons which was determined by confocal imaging their emission to argon laser excitation at 488 nm, after loading the cells with fluo-3. FIG. 6E shows the marked increase in fluo-3 fluorescence typical of the neuronal calcium response to K⁺-depolarization. FIGS. 6F-G shows that of 11 MAP-2+ cells in FIG. 6B, four were found to have incorporated 3H-thymidine+; two of these are shown after 3Hthymidine autoradiography in FIG. 6F (arrows, in phase), and after staining for MAP-2 in FIG. 6G. The remaining seven neurons in FIG. 6B may have been cells that committed to neuronal differentiation without intervening cell division.

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 TI EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS DERIVED THEREFROM
 IN Ben-Hur Tamir (IL); Pera Martin Frederick (AU); Reubinoff Benjamin Eithan (IL)
 PA Unassigned Or Assigned To Individual (68000)
 PI US 2002164308 AI 20021107
 AI US 2001-970543 20011004
 RLI US 2001-808382 20010314 CONTINUATION-IN-PART PENDING
 PRAI AU 2000-6211 20000314
 AU 2000-1279 20001106
 AU 2001-2920 20010206
 FI US 2002164308 20021107
 DT Utility; Patent Application - First Publication
 FS CHEMICAL
 APPLICATION
 CLMN 73
 GI 38 Figure(s).

FIG. 1 shows phase contrast micrographs of ES cells and their differentiated progeny. A, inner cell mass three days after plating. B, colony of ES cells. C, higher magnification of an area of an ES cell colony. D, an area of an ES cell colony undergoing spontaneous differentiation during routine passage. E, a colony four days after plating in the absence of a feeder cell layer but in the presence of 2000 units/ml human LIF undergoing differentiation in its periphery. F, neuronal cells in a high density culture. Scale bars: A and C, 25 microns; B and E, 100 microns; D and F, 50 microns.

FIG. 2 shows marker expression in ES cells and their differentiated somatic progeny. A, ES cell colony showing histochemical staining for alkaline phosphatase. B, ES cell colony stained with antibody MC-813-70 recognising the SSEA-4 epitope. C, ES cell colony stained with antibody TRAL-60. D, ES cell colony stained with antibody GCTM-2. E, high density culture, cell body and processes of a cell stained with antineurofilament 68 kDa protein. F, high density culture, cluster of cells and network of processes emanating from them stained with antibody against neural cell adhesion molecule. G, high density culture, cells showing cytoplasmic filaments stained with antibody to muscle actin. H, high density culture, cell showing cytoplasmic filaments stained with antibody to desmin. Scale bars: A, 100 microns; B-D, and F, 200 microns; E, G and H, 50 microns.

FIG. 3 shows RT-PCR analysis of gene expression in ES cells and their differentiated derivatives. All panels show 1.5% agarose gels stained with ethidium bromide. A, expression of Oct-4 and b-actin in ES stem cells and high density cultures. Lane 1, 100 bpDNA ladder. Lane 2, stem cell culture, b-actin. Lane 3, stem cell culture, Oct-4. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lane 5, high density culture, b-actin. Lane 6, high density culture, Oct-4. Lane 7, high density culture, PCR for Oct-4 carried out with omission of reverse transcriptase. b-actin band is 200 bp and Oct-4 band is 320 bp. B, expression of nestin and Pax-6 in neural progenitor cells that were derived from differentiating ES colonies. Left lane, 100 bp DNA ladder; lane 1, b-actin in HX 142 neuroblastoma cell line (positive control for nestin PCR); lane 2, b-actin in neural progenitor cells; lane 3, nestin in HX 142 neuroblastoma cell line; lane 4, nestin in neural progenitor cells; lane 5, nestin PCR on same sample

as lane 4 without addition of reverse transcriptase; lane 6, Pax-6; lane 7, Pax-6 PCR on same sample as lane 6 without addition of reverse transcriptase. Nestin band is 208 bp, Pax-6 is 274 bp. C, expression of glutamic acid decarboxylase in cultures of neurons. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, b-actin PCR on same sample as lane 1 without addition of reverse transcriptase; lane 3, glutamic acid decarboxylase; lane 4 glutamic acid decarboxylase on same sample as lane 3 without addition of reverse transcriptase. Glutamic acid decarboxylase band is 284 bp. D, expression of GABA A alpha 2 receptor. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, GABA A alpha 2 receptor; lane 3, PCR without addition of reverse transcriptase. GABA A alpha 2 receptor subunit band is 471 bp.

FIG. 4 shows histology of differentiated elements found in teratomas formed in the testis of SCID mice following inoculation of HES-1 or HES-2 colonies. A, cartilage and squamous epithelium, HES-2. B, neural rosettes, HES-2. C, ganglion, gland and striated muscle, HES-1. D, bone and cartilage, HES-1. E, glandular epithelium, HES-1. F, ciliated columnar epithelium, HES-1. Scale bars: A-E, 100 microns; F, 50 microns.

FIG. 5 shows phase contrast microscopy and immunochemical analysis of marker expression in neural progenitor cells isolated from differentiating ES cultures. A, phase contrast image of a sphere formed in serum-free medium. B-D, indirect immunofluorescence staining of spheres, 4 hours after plating on adhesive substrate, for N-CAM, nestin, and vimentin respectively. In C and D, cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining; confocal examination revealed that cells throughout the sphere were decorated by both antibodies. Scale bar is 100 microns in all panels.

FIG. 6 shows phase contrast appearance and marker expression in cultures of neurons derived from progenitor cells shown in FIG. 5. A, phase contrast micrograph of differentiated cells emanating from a sphere plated onto adhesive surface. B-H, indirect immunofluorescence microscopy of differentiated cells decorated with antibodies against 200 kDa neurofilament protein (B), 160 kDa neurofilament protein (C), MAP2a+b (D), glutamate (E), synaptophysin (F), glutamic acid decarboxylase (G) and beta-tubulin (H). Scale bars: A, ;B, 100 microns; C, 200 microns; D, 20 microns; E and F, 10 microns; G, 20 microns; H, 25 microns.

FIG. 7 shows neural precursors proliferating as a monolayer on a plastic tissue culture dish in the presence of EGF and ****bFGF****. These monolayer cultures of proliferating cells were obtained after prolonged cultivation (2-3 weeks) of the spheres in the presence of growth factors without sub-culturing.

FIG. 8 shows phase contrast appearance of a culture consisting of differentiated neural cells.

FIG. 9 shows phase contrast appearance of a sphere that is formed 72 hours after the transfer of a clump of undifferentiated ES cells into serum free medium (Scale bar 100 microns).

FIG. 10 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters that were generated from differentiating ES colonies and were propagated for 14-15 weeks were disaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 11 shows indirect immunofluorescence staining of a sphere, 4 hours after plating on adhesive substrate, for N-CAM. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 100 microns).

FIG. 12 shows indirect immunofluorescence membranous staining for N-CAM of single cells at the periphery of a sphere 4 hours after plating on adhesive substrate. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 13 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament nestin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 14 shows indirect immunofluorescence microscopy of a differentiated cell decorated with antibodies against the oligodendrocyte progenitor marker O4. (Scale bar 12.5 microns).

FIG. 15 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament vimentin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 7 passages. (Scale bar 25 microns).

FIG. 16 shows the growth pattern of spheres that were generated directly from undifferentiated ES cells. Each bar represents the mean (\pm SD) increment in volume per week of 24 spheres at first to sixteen weeks after derivation. A more excessive growth rate is evident during the first 5 weeks.

FIG. 17 shows persistent growth in the volume of spheres along time. Each bar represents the mean (\pm SD) increment in volume per week of 24 spheres at nine to twenty one weeks after derivation. The spheres were generated from differentiating ES colonies.

FIG. 18 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters, that were generated directly from undifferentiated ES cells and were propagated 5-7 weeks, were disaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 19 shows RT-PCR analysis of gene expression in ES cells (a week after passage) and neural spheres derived from differentiating colonies and directly from undifferentiated ES cell. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1, 2 and 3, Oct-4 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lanes 5, 6, and 7, nestin in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 8, stem cell culture, PCR for nestin carried out with omission of reverse transcriptase. Lanes 9, 10 and 11, Pax-6 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 12, stem cell culture, PCR for Pax-6 carried out with omission of reverse transcriptase. Lane 13, 100 bp DNA ladder. Oct-4 band is 320 bp, nestin is 208 bp and Pax-6 is 274 bp.

FIG. 20 shows indirect immunofluorescence microscopy of differentiated ***astrocyte*** cells decorated with antibody against GFAP. (Scale bar 25 microns).

FIG. 21 shows indirect immunofluorescence microscopy of brain sections of two mice (A and B) 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Cells with a nucleus decorated with anti BrDU (brown stain, black arrow) are evident near the ventricular surface (white arrow indicate mouse unstained nuclei, bar=20 microns).

FIG. 22 shows indirect immunofluorescence microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. wide spread distribution of transplanted human cells decorated by anti BrDU antibodies is evident in the periventricular areas. The periventricular area in A is demonstrated at a higher magnification in B and C. (Bars=150, 60 and 30 microns in A, B and C).

FIG. 23 shows indirect immunocytochemical microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. The transplanted human cells are migrating along the rostral migratory stream (bar=150 microns).

FIG. 24 shows RT-PCR analysis of gene expression in neural spheres derived from differentiating (A) and undifferentiated (B) ES cells. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1 and 10, 100 bpDNA ladder; Lane 2, CD34; Lane 3, Flk-1; lane4, HNF-3; lane 5, alfafetoprotein. Lanes 6-9 PCR reaction on the same samples as lanes 2-5 carried out with the omission of reverse transcriptase. CD-34 band is 200 bp, Flk-1 is 199, HNF-3 is 390, AFP is 340 bp.

FIG. 25 shows by RT-PCR analysis the expression of GFAP and the pip gene in differentiated cells from neural spheres derived from differentiating ES cell colonies. The expression of GFAP indicates differentiation into ***astrocytes*** while the presence of both dm-20 and pip transcripts indicate that differentiation into oligodendrocyte cells has occurred. Lanes 2, 4, 6 and lanes 3, 5, 7 are from two separate RNA samples from differentiated spheres that were independently derived from ES cells. Lane 1 and 8, 100 bp DNA ladder; Lanes 2 and 4, GFAP; lanes 3 and 5, plp and dm-20; lanes 6 and 7, PCR reaction on the same samples as lanes 3 and 5 carried out with the omission of reverse transcriptase. GFAP band is 383, pip band is 354 bp and dm-20 is 249 bp.

FIG. 26 shows a dark field stereomicroscopic photograph of areas (arrows) destined to give rise to neural precursors in a differentiating ES cell colony 3 weeks after passage (bar=1.6 mm).

FIG. 27 shows indirect immunochemical analysis of marker expression in cultures of neurons derived from progenitor cells that were derived directly from undifferentiated ES cells: A, indirect immunofluorescence microscopy of neurites decorated with antibody against 160 kDa neurofilament protein. B and C, indirect immunofluorescence staining of differentiated cells for MAP2a+b and beta-tubulin III. Scale bars: A 100 microns, B and C 10 microns.

FIG. 28 shows indirect immunochemical analysis of the expression of tyrosine hydroxylase. Neurites (A) and a differentiated cell (B) are decorated with antibodies against tyrosine hydroxylase. Scale bars: 30 microns.

FIG. 29 shows in vivo differentiation into ***astrocyte*** cells of transplanted ***human*** ***neural*** ***progenitors*** pre-labeled with BrdU. Donor cells are identified by indirect immunochemical detection of BrdU (dark nuclei, arrows). Dual staining demonstrates donor cells decorated by anti GFAP (orange). Transplanted cells are migrating into the brain parenchyma (white arrow) and are also found in the periventricular zone (dark arrow) (A). A higher magnification of cells that have differentiated into ***astrocytes*** and migrated into the host brain (B).

FIG. 30 shows in vivo differentiation into oligodendrocyte cells of transplanted ***human*** ***neural*** ***progenitors*** pre-labeled with BrdU. Donor cells are identified by indirect immunochemical detection of BrdU (dark nuclei, arrows). Dual staining demonstrates donor cells decorated by anti CNPase (orange).

FIG. 31 shows cumulative growth curve for ***human*** ***neural*** ***progenitors*** derived from differentiating colonies. (A) Continuous growth is evident during an 18-22 week period. The increment in the volume of the spheres was continuously monitored as an indirect measure of the increase in cell numbers. A linear positive correlation between the volume of the spheres and the number of cells within the spheres (B, insert) was maintained along cultivation. It supported the validity of monitoring the increment of sphere volume as an indirect indicator of cell proliferation.

FIG. 32 shows RT-PCR analysis of the expression of non-neural markers in human ES derived spheres. All panels show 2% agarose gels stained with ethidium bromide. The symbols + and - indicate whether the PCR reaction was performed with or without the addition of reverse transcriptase. A 1 Kb plus DNA ladder was used in all panels. beta-actin band is 291 bp, keratin is 780 bp, Flk-1 is 199 bp, CD34 is 200 bp, AC-133 is 200 bp, transferrin is 367 bp, amylase is 490 bp and alpha 1 anti trypsin is 360 bp.

FIG. 33 shows a phase contrast micrograph of differentiated cells growing out from a sphere 2 weeks after plating onto an adhesive surface and culture in the absence of growth factors. Scale bar is 200 μ m.

FIG. 34 shows RT-PCR analysis of the expression of neuronal and glial markers in differentiated cells originating from human ES derived neural spheres. All panels show 2% agarose gels stained with ethidium bromide. The symbols + and - indicate whether the PCR reaction was performed with or without the addition of reverse transcriptase. A 1 Kb plus DNA ladder was used in all panels. Plp and dm-20 bands are 354 bp and 249 bp respectively, MBP is 379 bp, GFAP is 383 bp, NSE is 254 bp and NF-M is 430 bp.

FIG. 35 shows indirect immunochemical analysis of the expression of serotonin (A) and GABA (B). Scale bars are 20 μ m.

FIG. 36 shows dissemination of transplanted BrdU+ human ES derived neural progenitor cells in the mouse host brain.

(A) At 2 days after transplantation most cells were found lining the ventricular wall. (B) After 4-6 weeks most cells had left the ventricles (V) and populated the corpus callosum (CC), fimbria (fim), internal capsule (i.c.). BrdU+ cells were not found in the striatum (str) or CA region of the hippocampus (hipp). (C) Chains of BrdU+ cells were found in the rostral migratory stream (RMS). (D) BrdU+ cells in the periventricular white matter. (E) Higher magnification of D, to show nuclear specific localization of BrdU.

FIG. 37 shows identification of the transplanted cells in the brain by human and neural-lineage specific markers. (A) A typical chain of transplanted cells in the corpus callosum, stained with human specific anti-mitochondrial antibody. The mitochondrial staining (green fluorescence) on Nomarsky background (blue, cell nuclei indicated by asterisk) shows a typical perinuclear localization. (B) Double staining for BrdU (green fluorescence) and human specific anti ribonuclear protein (red fluorescence) shows nuclear co-localization, indicating that BrdU+ cells were indeed of human origin. (C) A GFAP+ ***astrocyte*** (red) from the periventricular region, co-labeled with BrdU (green), indicating its origin from the graft. (D) An NG2+ oligodendrocyte progenitor (red) in the periventricular region, co-labeled with BrdU (green). (E) A CNPase+ oligodendrocyte (red) in the corpus callosum, co-labeled with BrdU (immunohistochemistry, shown as dark nucleus in Nomarsky). (F) Neuronal processes in the fimbria, stained with a human specific anti-70 kDa neurofilament. (G) A beta III-tubulin+ neuron (green fluorescence) in the olfactory bulb, co-labeled with BrdU (as dark nucleus (arrow) in Nomarsky). Bars=10 μ m. !

L4 ANSWER 9 OF 24 IFIPAT COPYRIGHT 2004 IFI on STN
 AN 10124433 IFIPAT;IFIUDB;IFICDB
 TI EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS DERIVED THEREFROM; SUCH
 AS NEURAL PROGENITOR CELLS CAPABLE OF GIVING RISE TO MATURE SOMATIC CELLS
 INCLUDING NEURAL CELLS AND/OR GLIAL CELLS RECOGNIZABLE BY EXPRESSION OF
 SPECIFIC MARKERS
 IN Ben-Hur Tamir (IL); Pera Martin Frederick (AU); Reubinoff Benjamin Eithan
 (IL)
 PA Unassigned Or Assigned To Individual (68000)
 PI US 2002068045 A1 20020606
 AI US 2001-808382 20010314
 PRAI AU 2000-6211 20000314
 AU 2000-1279 20001106
 AU 2001-2920 20010206
 FI US 2002068045 20020606
 DT Utility; Patent Application - First Publication
 FS CHEMICAL
 APPLICATION
 CLMN 85
 GI 30 Figure(s).

FIG. 1 shows phase contrast micrographs of ES cells and their differentiated progeny. A, inner cell mass three days after plating. B, colony of ES cells. C, higher magnification of an area of an ES cell colony. D, an area of an ES cell colony undergoing spontaneous differentiation during routine passage. E, a colony four days after plating in the absence of a feeder cell layer but in the presence of 2000 units/ml human LIF undergoing differentiation in its periphery. F, neuronal cells in a high density culture. Scale bars: A and C, 25 microns; B and E, 100 microns; D and F, 50 microns.

FIG. 2 shows marker expression in ES cells and their differentiated somatic progeny. A, ES cell colony showing histochemical staining for alkaline phosphatase. B, ES cell colony stained with antibody MC-813-70 recognising the SSEA-4 epitope. C, ES cell colony stained with antibody TRA1-60. D, ES cell colony stained with antibody GCTM-2. E, high density culture, cell body and processes of a cell stained with antineurofilament 68 kDa protein. F, high density culture, cluster of cells and network of processes emanating from them stained with antibody against neural cell adhesion molecule. G, high density culture, cells showing cytoplasmic filaments stained with antibody to muscle actin. H, high density culture, cell showing cytoplasmic filaments stained with antibody to desmin. Scale bars: A, 100 microns; B-D, and F, 200 microns; E, G and H, 50 microns.

FIG. 3 shows RT-PCR analysis of gene expression in ES cells and their differentiated derivatives. All panels show 1.5% agarose gels stained with ethidium bromide. A, expression of Oct-4 and b-actin in ES stem cells and high density cultures. Lane 1, 100 bpDNA ladder. Lane 2, stem cell culture, b-actin. Lane 3, stem cell culture, Oct-4. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lane 5, high density culture, b-actin. Lane 6, high density culture, Oct-4. Lane 7, high density culture, PCR for Oct-4 carried out with omission of reverse transcriptase. b-actin band is 200 bp and Oct-4 band is 320 bp. B, expression of nestin and Pax-6 in neural progenitor cells that were derived from differentiating ES colonies. Left lane, 100 bp DNA ladder; lane 1, b-actin in HX 142 neuroblastoma cell line (positive control for nestin PCR); lane 2, b-actin in neural progenitor cells; lane 3, nestin in HX 142 neuroblastoma cell line; lane 4, nestin in neural progenitor cells; lane 5, nestin PCR on same sample as lane 4 without addition of reverse transcriptase; lane 6, Pax-6; lane 7, Pax-6 PCR on same sample as line 6 without addition of reverse transcriptase. Nestin band is 208 bp, Pax-6 is 274 bp. C, expression of glutamic acid decarboxylase in cultures of neurons. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, b-actin PCR on same sample as lane 1 without addition of reverse transcriptase; lane 3, glutamic acid decarboxylase; lane 4 glutamic acid decarboxylase on same sample as lane 3 without addition of reverse transcriptase. Glutamic acid decarboxylase band is 284 bp. D, expression of GABA A alpha 2 receptor. Left lane, 100 bp DNA ladder; lane 1, b-actin; lane 2, GABA A alpha 2 receptor; lane 3, PCR without addition of reverse transcriptase. GABA A alpha 2 receptor subunit band is 471 bp.

FIG. 4 shows histology of differentiated elements found in teratomas formed in the testis of SCID mice following inoculation of HES-1 or HES-2 colonies. A, cartilage and squamous epithelium, HES-2. B, neural rosettes, HES-2. C, ganglion, gland and striated muscle, HES-1. D, bone and cartilage, HES-1. E, glandular epithelium, HES-1. F, ciliated columnar epithelium, HES-1. Scale bars: A-E, 100 microns; F, 50 microns.

FIG. 5 shows phase contrast microscopy and immunochemical analysis of

marker expression in neural progenitor cells isolated from differentiating ES cultures. A, phase contrast image of a sphere formed in serum-free medium. B-D, indirect immunofluorescence staining of spheres, 4 hours after plating on adhesive substrate, for N-CAM, nestin, and vimentin respectively. In C and D, cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining; confocal examination revealed that cells throughout the sphere were decorated by both antibodies. Scale bar is 100 microns in all panels.

FIG. 6 shows phase contrast appearance and marker expression in cultures of neurons derived from progenitor cells shown in FIG. 5. A, phase contrast micrograph of differentiated cells emanating from a sphere plated onto adhesive surface. B-H, indirect immunofluorescence microscopy of differentiated cells decorated with antibodies against 200 kDa neurofilament protein (B), 160 kDa neurofilament protein (C), MAP2a+b (D), glutamate (E), synaptophysin (F), glutamic acid decarboxylase (G) and beta-tubulin (H). Scale bars: A, B, 100 microns; C, 200 microns; D, 20 microns; E and F, 10 microns; G, 20 microns; H, 25 microns.

FIG. 7 shows neural precursors proliferating as a monolayer on a plastic tissue culture dish in the presence of EGF and *****bFGF*****. These monolayer cultures of proliferating cells were obtained after prolonged cultivation (2-3 weeks) of the spheres in the presence of growth factors without sub-culturing.

FIG. 8 shows phase contrast appearance of a culture consisting of differentiated neural cells.

FIG. 9 shows phase contrast appearance of a sphere that is formed 72 hours after the transfer of a clump of undifferentiated ES cells into serum free medium (Scale bar 100 microns).

FIG. 10 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters that were generated from differentiating ES colonies and were propagated for 14-15 weeks were disaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 11 shows indirect immunofluorescence staining of a sphere, 4 hours after plating on adhesive substrate, for N-CAM. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 100 microns).

FIG. 12 shows indirect immunofluorescence membranous staining for N-CAM of single cells at the periphery of a sphere 4 hours after plating on adhesive substrate. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of the resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 13 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament nestin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 5 passages. (Scale bar 25 microns).

FIG. 14 shows indirect immunofluorescence microscopy of a differentiated cell decorated with antibodies against the oligodendrocyte progenitor marker O4. (Scale bar 12.5 microns).

FIG. 15 shows indirect immunofluorescence staining of a sphere 4 hours after plating on adhesive substrate for the intermediate filament vimentin. Cells at the base of the sphere were placed in plane of focus to illustrate filamentous staining. The sphere was generated by direct transfer of undifferentiated ES cells into serum free medium and propagation of resulting spheres for 7 passages. (Scale bar 25 microns).

FIG. 16 shows the growth pattern of spheres that were generated directly from undifferentiated ES cells. Each bar represents the mean (+SD) increment in volume per week of 24 spheres at first to twelve weeks after derivation. A more excessive growth rate is evident during the first 5 weeks.

FIG. 17 shows persistent growth in the volume of spheres along time. Each bar represents the mean (+SD) increment in volume per week of 24 spheres at nine to twenty one weeks after derivation. The spheres were generated from differentiating ES colonies.

FIG. 18 shows linear correlation between the volume of spheres and the number of progenitor cells within a sphere. Spheres of various diameters, that were generated directly from undifferentiated ES cells and were propagated 5-7 weeks, were disaggregated into single cell suspension and the number of cells per sphere was counted.

FIG. 19 shows RT-PCR analysis of gene expression in ES cells (a week after passage) and neural spheres derived from differentiating colonies and directly from undifferentiated ES cell. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1, 2 and 3, Oct-4 in ES cell culture, neural spheres derived from differentiating colonies, neural

spheres derived from undifferentiated ES cells. Lane 4, stem cell culture, PCR for Oct-4 carried out with omission of reverse transcriptase. Lanes 5, 6, and 7, nestin in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 8, stem cell culture, PCR for nestin carried out with omission of reverse transcriptase. Lanes 9, 10 and 11, Pax-6 in ES cell culture, neural spheres derived from differentiating colonies, neural spheres derived from undifferentiated ES cells. Lane 12, stem cell culture, PCR for Pax-6 carried out with omission of reverse transcriptase. Lane 13, 100 bp DNA ladder. Oct-4 band is 320 bp, nestin is 208 bp and Pax-6 is 274 bp.

FIG. 20 shows indirect immunofluorescence microscopy of differentiated *****astrocyte***** cells decorated with antibody against GFAP. (Scale bar 25 microns).

FIG. 21 shows indirect immunofluorescence microscopy of brain sections of two mice (A and B) 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Cells with a nucleus decorated with anti BrDU (brown stain, black arrow) are evident near the ventricular surface (white arrow indicate mouse unstained nuclei, bar=20 microns).

FIG. 22 shows indirect immunofluorescence microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. Wide spread distribution of transplanted human cells decorated by anti BrDU antibodies is evident in the periventricular areas. The periventricular area in A is demonstrated at a higher magnification in B and C. (Bars=150, 60 and 30 microns in A, B and C).

FIG. 23 shows indirect immunocytochemical microscopy of brain sections of a mice 4 weeks after transplantation of human neural precursors prelabeled with BrDU. The transplanted human cells are migrating along the rostral migratory stream (bar=150 microns).

FIG. 24 shows RT-PCR analysis of gene expression in neural spheres derived from differentiating (A) and undifferentiated (B) ES cells. All panels show 2% agarose gels stained with ethidium bromide. Lanes 1 and 10, 100 bpDNA ladder; Lane 2, CD34; Lane 3, Flk-1; lane 4, HNF-3; lane 5, alfafetoprotein. Lanes 6-9 PCR reaction on the same samples as lanes 2-5 carried out with the omission of reverse transcriptase. CD-34 band is 200 bp, Flk-1 is 199, HNF-3 is 390, AFP is 340 bp.

FIG. 25 shows by RT-PCR analysis the expression of GFAP and the plp gene in differentiated cells from neural spheres derived from differentiating ES cell colonies. The expression of GFAP indicates differentiation into *****astrocytes***** while the presence of both dm-20 and plp transcripts indicate that differentiation into oligodendrocyte cells has occurred. Lanes 2,4,6 and lanes 3,5,7 are from two separate RNA samples from differentiated spheres that were independently derived from ES cells. Lane 1 and 8, 100 bp DNA ladder; Lanes 2 and 4, GFAP; lanes 3 and 5, plp and dm-20; lanes 6 and 7, PCR reaction on the same samples as lanes 3 and 5 carried out with the omission of reverse transcriptase. GFAP band is 383, plp band is 354 bp and dm-20 is 249 bp.

FIG. 26 shows a dark field stereomicroscopic photograph of areas (arrows) destined to give rise to neural precursors in a differentiating ES cell colony 3 weeks after passage (bar=1.6 mm).

FIG. 27 shows indirect immunochemical analysis of marker expression in cultures of neurons derived from progenitor cells that were derived directly from undifferentiated ES cells: A, indirect immunofluorescence microscopy of neurites decorated with antibody against 160 kDa neurofilament protein. B and C, indirect immunofluorescence staining of differentiated cells for MAP2a+b and beta-tubulin III. Scale bars: A 100 microns, B and C 10 microns.

FIG. 28 shows indirect immunochemical analysis of the expression of tyrosine hydroxylase. Neurites (A) and a differentiated cell (B) are decorated with antibodies against tyrosine hydroxylase. Scale bars: 30 microns.

FIG. 29 shows in vivo differentiation into *****astrocyte***** cells of transplanted *****human***** *****neural***** *****progenitors***** prelabeled with BrDU. Donor cells are identified by indirect immunochemical detection of BrDU (dark nuclei, arrows). Dual staining demonstrates donor cells decorated by anti GFAP (orange). Transplanted cells are migrating into the brain parenchyma (white arrow) and are also found in the periventricular zone (dark arrow) (A), A higher magnification of cells that have differentiated into *****astrocytes***** and migrated into the host brain (B).

FIG. 30 shows in vivo differentiation into oligodendrocyte cells of transplanted *****human***** *****neural***** *****progenitors***** prelabeled with BrDU. Donor cells are identified by indirect immunochemical detection of BrDU (dark nuclei, arrows). Dual staining demonstrates donor cells decorated by anti CNPase (orange). !

ACCESSION NUMBER: 1998:578539 PROMT
 TITLE: CytoTherapeutics Researchers Demonstrate Potential for
 Human Neural Stem Cells to Repair or Replace CNS Tissue.
 SOURCE: Business Wire, (9 Nov 1998) pp. 1351.
 LANGUAGE: English
 WORD COUNT: 1701
 FULL TEXT IS AVAILABLE IN THE ALL FORMAT

L4 ANSWER 11 OF 24 USPATFULL on STN

AN 2004:51457 USPATFULL
 TI Functional role and potential therapeutic use of PACAP, VIP and
 Maxadilan in relation to adult neural stem or progenitor cells
 IN Mercer, Alex, Bromma, SWEDEN
 Patrone, Cesare, Hagersten, SWEDEN
 Ronnholm, Harriet, Trangsund, SWEDEN
 Wikstrom, Lilian, Spanga, SWEDEN
 PI US 2004038888 A1 20040226
 AI US 2003-429062 A1 20030502 (10)
 PRAI US 2002-377734P 20020503 (60)
 US 2002-393264P 20020702 (60)
 US 2002-426827P 20021115 (60)
 DT Utility
 FS APPLICATION
 LN.CNT 3987
 INCL INCLM: 514/012.000
 NCL NCLM: 514/012.000
 IC [7]
 ICM: A61K038-17

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 12 OF 24 USPATFULL on STN

AN 2004:13072 USPATFULL
 TI Genetically-modified neural progenitors and uses thereof
 IN Sabate, Olivier, Paris, FRANCE
 Horellou, Philippe, Paris, FRANCE
 Buc-Caron, Marie-Helene, Paris, FRANCE
 Mallet, Jacques, Paris, FRANCE
 PA Rhone-Poulenc Rorer S.A. (non-U.S. corporation)
 PI US 2004009592 A1 20040115
 AI US 2002-305386 A1 20021127 (10)
 RLI Continuation of Ser. No. US 1997-810315, filed on 28 Feb 1997, ABANDONED
 PRAI US 1996-12635P 19960301 (60)
 DT Utility
 FS APPLICATION
 LN.CNT 1050
 INCL INCLM: 435/368.000
 NCL NCLM: 435/368.000
 IC [7]
 ICM: C12N005-08

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 13 OF 24 USPATFULL on STN

AN 2004:7427 USPATFULL
 TI Potential growth factors from the human tumour cell line ht 1080
 IN Minger, Stephen L., London, UNITED KINGDOM
 Adams, Gregor, London, UNITED KINGDOM
 Francis, Paul, London, UNITED KINGDOM
 McClure, Myra, London, UNITED KINGDOM
 PI US 2004005661 A1 20040108
 AI US 2003-344503 A1 20030708 (10)
 WO 2001-GB3523 20010806
 PRAI GB 2000-19705 20000810
 DT Utility
 FS APPLICATION
 LN.CNT 1664
 INCL INCLM: 435/069.100
 INCLS: 435/226.000; 435/320.100; 435/366.000; 530/350.000; 536/023.200
 NCL NCLM: 435/069.100
 NCLS: 435/226.000; 435/320.100; 435/366.000; 530/350.000; 536/023.200
 IC [7]
 ICM: C12N009-64
 ICS: C07H021-04; C12N005-08; C07K014-47; C12P021-02

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 14 OF 24 USPATFULL on STN
AN 2003:312269 USPATFULL
TI Stem cell-like cells
IN Kruijer, Wiebe, Leusden, NETHERLANDS
PI US 2003219866 A1 20031127
AI US 2003-349505 A1 20030121 (10)
RLI Continuation of Ser. No. WO 2001-NL561, filed on 20 Jul 2001, UNKNOWN
PRAI EP 2000-202634 20000721
DT Utility
FS APPLICATION
LN.CNT 1309
INCL INCLM: 435/069.100
INCLS: 435/320.100; 435/366.000; 530/350.000; 536/023.500
NCL NCLM: 435/069.100
NCLS: 435/320.100; 435/366.000; 530/350.000; 536/023.500
IC [7]
ICM: C07K014-475
ICS: C07H021-04; C12P021-02; C12N005-08
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 15 OF 24 USPATFULL on STN
AN 2003:237328 USPATFULL
TI Functional role and potential therapeutic use of Reelin, Gas6 and Protein S in relation to adult neural stem or progenitor cells
IN Bertilsson, Goran, Vasterhaninge, SWEDEN
Falk, Anna, Solna, SWEDEN
Frisen, Jonas, Stockholm, SWEDEN
Heidrich, Jessica, Arsta, SWEDEN
Hellstrom, Kristina, Sodertalje, SWEDEN
Kortesmaa, Jarkko, Stockholm, SWEDEN
Lindquist, Per, Bromma, SWEDEN
Lundh, Hanna, Solna, SWEDEN
McGuire, Jacqueline, Huddinge, SWEDEN
Mercer, Alex, Bromma, SWEDEN
Patrone, Cesare, Hagersten, SWEDEN
Ronnholm, Harriet, Trangsund, SWEDEN
Wikstrom, Lilian, Spanga, SWEDEN
Zachrisson, Olof, Spanga, SWEDEN
PI US 2003165485 A1 20030904
AI US 2002-291171 A1 20021108 (10)
PRAI US 2001-344725P 20011109 (60)
US 2002-393263P 20020702 (60)
US 2001-345064P 20011109 (60)
US 2002-394397P 20020708 (60)
DT Utility
FS APPLICATION
LN.CNT 3554
INCL INCLM: 424/094.600
INCLS: 424/146.100
NCL NCLM: 424/094.600
NCLS: 424/146.100
IC [7]
ICM: A61K038-46
ICS: A61K039-395
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 16 OF 24 USPATFULL on STN
AN 2003:172722 USPATFULL
TI Compositions and methods for isolation, propagation, and differentiation of human stem cells and uses thereof
IN Neuman, Toomas, Santa Monica, CA, UNITED STATES
Levesque, Michel, Beverly Hills, CA, UNITED STATES
PI US 2003118566 A1 20030626
AI US 2002-216677 A1 20020808 (10)
PRAI US 2001-310727P 20010808 (60)
US 2001-312714P 20010816 (60)
DT Utility
FS APPLICATION
LN.CNT 1836
INCL INCLM: 424/093.210
INCLS: 424/093.700; 435/368.000
NCL NCLM: 424/093.210
NCLS: 424/093.700; 435/368.000
IC [7]
ICM: A61K048-00
ICS: C12N005-08

L4 ANSWER 17 OF 24 USPATFULL on STN
AN 2003:57459 USPATFULL
TI Isolation of neural stem cells using gangliosides and other surface markers
IN Klassen, Henry, Pasadena, CA, UNITED STATES
Schwartz, Michael, Garden Grove, CA, UNITED STATES
Young, Michael J., Gloucester, MA, UNITED STATES
PI US 2003040023 A1 20030227
AI US 2002-128009 A1 20020422 (10)
PRAI US 2001-285407P 20010420 (60)
DT Utility
FS APPLICATION
LN.CNT 1388
INCL INCLM: 435/007.210
INCLS: 435/368.000
NCL NCLM: 435/007.210
NCLS: 435/368.000
IC [7]
ICM: G01N033-567
ICS: C12N005-08

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 18 OF 24 USPATFULL on STN
AN 2003:37685 USPATFULL
TI Method for facilitating the production of differentiated cell types and tissues from embryonic and adult pluripotent and multipotent cells
IN Lanza, Robert, Clinton, MA, UNITED STATES
West, Michael, Boston, MA, UNITED STATES
PI US 2003027330 A1 20030206
AI US 2002-112939 A1 20020402 (10)
PRAI US 2001-280138P 20010402 (60)
DT Utility
FS APPLICATION
LN.CNT 1650
INCL INCLM: 435/366.000
INCLS: 435/455.000
NCL NCLM: 435/366.000
NCLS: 435/455.000
IC [7]
ICM: C12N005-08
ICS: C12N015-85

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 19 OF 24 USPATFULL on STN
AN 2002:301223 USPATFULL
TI Method of isolating human neuroepithelial precursor cells from human fetal tissue
IN Mayer-Proschel, Margot, Pittsford, NY, UNITED STATES
Rao, Mahendra S., Salt Lake City, UT, UNITED STATES
Tresco, Patrick A., Sandy, UT, UNITED STATES
Messina, Darin J., Salt Lake City, UT, UNITED STATES
PI US 2002168767 A1 20021114
AI US 2001-813429 A1 20010321 (9)
DT Utility
FS APPLICATION
LN.CNT 829
INCL INCLM: 435/368.000
INCLS: 800/008.000
NCL NCLM: 435/368.000
NCLS: 800/008.000
IC [7]
ICM: C12N005-08
ICS: A01K067-00

L4 ANSWER 20 OF 24 USPATFULL on STN
AN 2002:92658 USPATFULL
TI Compositions and methods for treatment of mitochondrial diseases
IN Von Borstel, Reid W., Potomac, MD, UNITED STATES
Saydoff, Joel A., Middletown, MD, UNITED STATES
PI US 2002049182 A1 20020425
AI US 2001-930494 A1 20010816 (9)
RLI Continuation-in-part of Ser. No. US 2001-763955, filed on 28 Feb 2001,
PENDING A 371 of International Ser. No. WO 1999-US19725, filed on 31 Aug 1999, UNKNOWN Continuation-in-part of Ser. No. US 1998-144096, filed on 31 Aug 1998, PENDING

DT Utility
FS APPLICATION
LN.CNT 2171
INCL INCLM: 514/044.000
INCLS: 514/051.000
NCLM: 514/044.000
NCLS: 514/051.000
IC [7]
ICM: A61K048-00
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 21 OF 24 USPATFULL on STN
AN 2002:66630 USPATFULL
TI Methods of transducing neural cells using lentivirus vectors
IN Davidson, Beverly L., North Liberty, IA, UNITED STATES
Alisky, Joseph M., Iowa City, IA, UNITED STATES
Dubensky, Thomas W., JR., Piedmont, CA, UNITED STATES
Hughes, Stephanie M., Iowa City, IA, UNITED STATES
Jolly, Douglas, Encinitas, CA, UNITED STATES
Sauter, Sybille L., Del Mar, CA, UNITED STATES
PI US 2002037281 A1 20020328
AI US 2001-866532 A1 20010525 (9)
PRAI US 2000-207541P 20000526 (60)
US 2001-279035P 20010327 (60)

DT Utility
FS APPLICATION
LN.CNT 1641
INCL INCLM: 424/093.210
INCLS: 435/456.000; 435/368.000
NCLM: 424/093.210
NCLS: 435/456.000; 435/368.000
IC [7]
ICM: C12N015-867
ICS: A61K048-00; C12N005-08
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 22 OF 24 USPATFULL on STN
AN 2002:22068 USPATFULL
TI Method for isolating and purifying multipotential neural progenitor
cells and multipotential neural progenitor cells
IN Goldman, Steven A., South Salem, NY, UNITED STATES
Okano, Hideyuki, Osaka, JAPAN
PI US 2002012903 A1 20020131
AI US 2000-747810 A1 20001222 (9)
PRAI US 1999-173003P 19991223 (60)

DT Utility
FS APPLICATION
LN.CNT 2350
INCL INCLM: 435/004.000
INCLS: 435/368.000
NCLM: 435/004.000
NCLS: 435/368.000
IC [7]
ICM: C12Q001-00
ICS: C12N005-08
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 23 OF 24 USPATFULL on STN
AN 2002:12280 USPATFULL
TI GENETICALLY-MODIFIED NEURAL PROGENITORS AND USES THEREOF
IN SABATE, OLIVIER, PARIS, FRANCE
HORELLOU, PHILIPPE, PARIS, FRANCE
BUC-CARON, MARIE-HELENE, PARIS, FRANCE
MALLET, JACQUES, PARIS, FRANCE
PA Rhone-Poulenc Rorer, S.A. (non-U.S. corporation)
PI US 2002006660 A1 20020117
AI US 1997-810315 A1 19970228 (8)
PRAI US 1996-12635P 19960301 (60)

DT Utility
FS APPLICATION
LN.CNT 1048
INCL INCLM: 435/325.000
INCLS: 514/044.000
NCLM: 435/325.000
NCLS: 514/044.000
IC [7]

ICM: C12N005-02

ICS: A61K031-70

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 24 OF 24 USPATFULL on STN
AN 1999:117338 USPATFULL
TI Engraftable human neural stem cells
IN Snyder, Evan Y., Jamaica Plain, MA, United States
wolfe, John H., Philadelphia, PA, United States
Kim, Seung U., Vancouver, Canada
PA The Children's Medical Center Corp., Boston, MA, United States (U.S.
corporation)
PI US 5958767 19990928
AI US 1998-133873 19980814 (9)
DT Utility
FS Granted
LN.CNT 1267
INCL INCLM: 435/368.000
INCLS: 435/455.000
NCL NCLM: 435/368.000
NCLS: 435/455.000
IC [6]
ICM: C12N005-08
EXF 935/325; 935/366; 935/368; 935/455
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
STN INTERNATIONAL LOGOFF AT 16:00:13 ON 23 APR 2004